AD_____

Award Number:	W81XWH-10-2-0092
TITLE:	Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress
PRINCIPAL INVESTIGATOR:	Stephen Ahlers
CONTRACTING ORGANIZATION:	The Geneva Foundation Tacoma, WA 98402
REPORT DATE:	September 2014
TYPE OF REPORT:	Final
PREPARED FOR:	U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT:

☒ Approved for public release; distribution unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
September 2014	Final	1 Sept 2010 -31 August 2014

Neurocognitive and Biomarker Evaluation of Combination mTBI from Blast Overpressure and Traumatic Stress

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-10-2-0092

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Stephen Ahlers Ph.D. ,Raymond Genovese, Ph.D., Jitandra Dave, Ph.D.

email: lwagner@genevausa.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Geneva Foundation

Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Mild traumatic brain injury (mTBI) and post-traumatic stress disorder (PTSD) are major medical issues for the Warfighter. The current project is designed to evaluate the impact of mild traumatic brain injury (using blast overpressure) and the processes involved in traumatic stress (using a predator exposure procedure and a conditioned fear procedure) in a rodent model. The studies evaluate these insults alone and in combination to specifically address the question of whether mTBI can exacerbate the effects of psychological stress. Additionally, following the insults, a molecular biological evaluation is performed based upon the discovery of biomarkers that have been shown to be correlated with other forms of TBI. Thus, the project aims to systematically assess the combined effects of blast overpressure, traumatic stress and learned stress responses in rodents with the aim of understanding how these forces may interact to impact behavior as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation.

15. SUBJECT TERMS
Nothing Listed

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**	UU	10	**19b. TELEPHONE NUMBER** *(include area code)*
U	U	U			

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

INTRODUCTION:

There is a high co-morbidity of mild traumatic brain injury (mTBI) and post traumatic stress disorder (PTSD) in Warfighters. Co-morbid mTBI and PTSD appears to be more prevalent than mTBI cases in isolation. Mild TBI and PTSD are statistically ranked the highest of battlefield injuries in OIF and OEF. It is generally assumed that the manifestation of mTBI symptoms result from one or more exposures to improvised explosive devices (IEDs) and that PTSD symptoms result from exposure to prolonged battlefield stress. The high incidence and comorbidity of PTSD and mTBI underscore an imperative for the DoD research community to gain an understanding of the underlying mechanisms that precipitate these conditions together with the often associated post-concussive syndrome (PCS) which appears to share many of the same cognitive and emotive symptoms associated with TBI and PTSD.

KEYWORDS
stress, stress disorders, posttraumatic-stress-disorders, post-traumatic-stress-disorder animal model, PTSD, rat, blast injuries, brain injuries, fear, anxiety, biological marker, biomarker, protein, proteins, combat, war, trauma, traumatic brain injury, neurotrauma, proteomics, 2-D DIGE, mass spectroscopy, pain, CSF, urine, blast, blast overpressure, brain injury.

OVERALL PROJECT SUMMARY.

The purpose of the proposed experiments is to determine the relative contributions of repeated exposure to blast overpressure (BOP) and exposure to stressful (predatory) events, when presented alone and in combination, in a rodent model. The level of BOP used in the proposed experiments has been demonstrated by the PI (Ahlers) to be associated with mild outcomes where there is evidence of cognitive impairment in the absence of demonstrable pathology. The proposed experiments take advantage of years of extensive experience from the primary investigators (Ahlers & Genovese) in studies of the effects of BOP and stressful events and their effects on behavior. The assessment behavioral outcomes resulting from exposure to BOP and stress will be complemented by the assessment of the potential protein biomarkers by Dr. Dave and his group who have considerable experience identifying protein biomarkers for brain injury.

The objective of this research proposal is to systematically assess the combined effects of BOP and exposure to traumatic stress in rodents with the aim of understanding how these forces may interact with the manifestation of cognitive and emotive dysfunction, as well as evaluating their outcome on known biomarkers involved in TBI and stress response system activation.

Specific Aims

- Specific Aim 1: Assess the effects of repeated exposure to BOP and stress on cognitive and emotional performance. The primary investigator for this aim is Dr. Ahlers with support (predator exposures and performing the elevated plus maze)

from Dr. Genovese.

- Specific Aim 2: To characterize the extent to which BOP will specifically modify the process of conditioned fear in rats. The primary investigator for this aim is Dr. Genovese with support (blast exposures) from Dr. Ahlers.

- Specific Aim 3: Evaluate the combined effects of repeated exposure to BOP and stress on established biomarkers of traumatic brain injury (TBI). Primary investigator is Dr. Dave working in tandem with Drs. Genovese and Ahlers. Dr. Dave's effort is structurally aligned with Dr. Genovese's effort, as they are both WRAIR performers.

KEY RESEARCH ACCOMPLISHMENTS: (Ahlers portion) We completed the study to assess the effects of combined blast and stress exposures on spatial working memory using a Morris water maze and the experiment using the elevated plus maze. The results of either study did not demonstrate any differences on the acquisition of learning the spatial location or anxiety for any of the experimental conditions leading to the conclusion that, at least within the parameters of the current study, the addition of a stressful experience to exposures to blast overpressure does not have an additive effect in impairing performance or manifesting anxiety when the assessment occurs shortly after the exposures. As we have noted in previous published work, there is evidence that the effects of blast and stress do not appear to become manifest until some weeks or months after the exposures, particularly the blast exposure, particularly those that involve the demonstration of anxiety. We noted in previous reports that there is evidence for the cellular machinery reflecting brain damage to the prefrontal and cortical areas of the brain in rats exposed to 3 x 75 kPa BOP. Crucially, the evidence suggests that BOP exposure may increase markers of apoptosis, programmed cell death. Apoptosis processes and consequent cellular death and reorganizational processes make take a long period to take hold after blast, thus explaining why there are long-term effects of blast and stress but not short-term. We expect that the biomarker analysis on samples from rats exposed to blast in these studies will provide a clue to the potential for long-term outcomes.

For the first phase of the study the co-PI role was to provide animals exposed to blast overpressure and to assist in the preparation of the animals and tissue for the biomarker portion of the experiment. The major portion of the work beyond the above was the conditioned fear experiments performed by Gr. Genovese's laboratory. These data were reported in the reports submitted by Dr. Genovese and will not be repeated here.

For the second phase of the study there are two experimental themes. One, the assessment of combined blast and stress exposures on spatial cognitive performance using procedures and parameters that we have previously demonstrated impairment of performance with BOP exposure alone (Ahlers, et. al., 2012). The second series of experiments assessed the combined blast and stress exposures on measures of anxiety using the elevated plus maze.

Assessment of the effects of repeated BOP and stress exposure on cognition (spatial working memory) using the Morris Water Maze (MWM)

In this experiment we assessed the effects of repeated exposure to BOP and stress on cognition (spatial working memory) using the Morris Water Maze (MWM) task previously described as sensitive to detect cognitive impairment after exposure to 3 BOP exposures, one per day, on three consecutive days. Four treatment groups are proposed as defined in Table 1 below. For this experiment we utilized the BOP and MWM parameters previously described where slight impairment of acquisition was observed in four block trials after exposure to three BOP exposures (one per day under anesthesia) at the 75 kPa BOP intensity where rats are facing the blast wave inside the WRAIR shock tube. To assess the effects of explicit stress and BOP an additional group of rats were exposed to the BOP and to a different predator stress on three consecutive days. 24 hours after the behavioral tests animals will be euthanized and tissue samples will be taken for biomarker analysis. A summary of the experimental conditions is provided below:

Condition	n	Predator exposures	BOP	Dependent Measures	
				MWM	Biomarkers
Control-Sham	10	-	-	x	x
Control-Predator	10	x	-	x	x
Blast-Sham	10	-	x	x	x
Blast-Predator	10	x	x	x	x

Table 1. Experimental design to assess repeated exposures to BOP and stress on spatial working memory.

In the basic 4 x 4 balanced experimental design shown above rats in the control (sham) condition were exposed to the BOP device without experiencing the blast and will also be exposed to a sham predator environment without explicit exposure to the predators. Behavioral assessment occurred 24 hours after the last exposure to BOP/stress or sham condition(s).

The results of the experiment are provided below. Figure 1 depicts the latency to find the sunken platform over the course of 4 blocked trials in which each block represented 4 trials. The four conditions outlined in the table above conditions are depicted. The solid black line depicts the control-sham condition. Over the course of the blocked trials rats in the control condition reached asymptotic performance by block three with only marginal improvement in latencies from block 3 to block 4.

There were no significant statistical differences between the four treatment conditions across the blocked trials despite the observation that the blast-predator condition appeared to acquire the task at a slower rate and to a lesser extent that the other treatment conditions. It may be that greater statistical power to distinguish the conditions may have been reached with additional animals. In our experience however, treatment

effects with group sizes of 10 are not likely to manifest with higher numbers of animals per group. Additionally, based upon our prior experience and power calculations, 10 animals per group are adequate to see treatment effects using this paradigm. It is also noteworthy that there was not a distinct impairment in the blast group compared to the sham condition, which represents a failure to replicate previous (unpublished) findings of a slight impairment of performance with the 3 x 75 kPa blast exposure regimen.

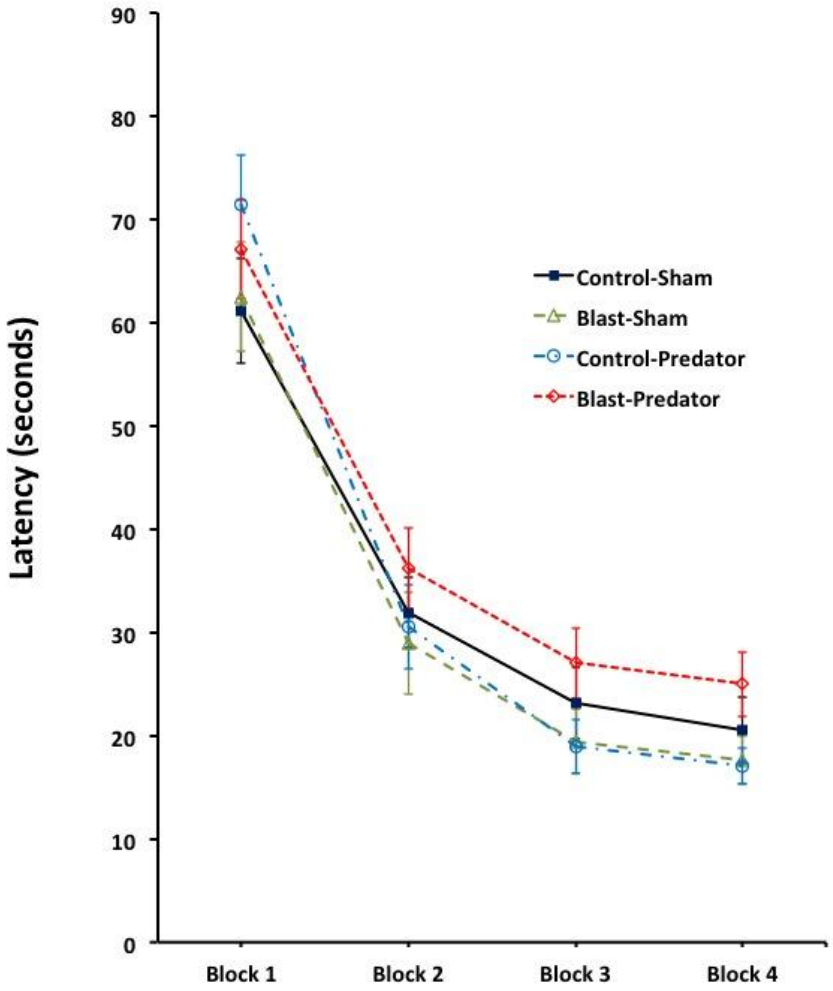

Figure 1. Effects of Repeated BOP and predator exposure on the Morris Water Maze, a test of working memory: Mean latency time to find the submerged platform in four blocked trials presented in a single day.

Assessment of the effects of repeated BOP and stress exposure on anxiety using the Elevated Plus Maze (EPM)

In this experiment we assessed the effects of repeated exposure to BOP and stress on on anxiety using the Elevated Plus Maze (EPM). Four treatment groups are proposed as defined in Table 2 below. For this experiment we utilized the BOP and MWM parameters previously described where slight impairment of acquisition was observed in four block trials after exposure to three BOP exposures (one per day under anesthesia) at the 75 kPa BOP intensity where rats are facing the blast wave inside the WRAIR shock tube. To assess the effects of explicit stress and BOP an additional group of rats were exposed to the BOP and to a different predator stress on three consecutive days. 24 hours after the behavioral tests animals will be euthanized and tissue samples will be taken for biomarker analysis. A summary of the experimental conditions is provided below:

Condition	n	Predator exposures	BOP	Dependent Measures	
				EPM	Biomarkers
Control-Sham	10	-	-	x	x
Control-Predator	10	x	-	x	x
Blast-Sham	10	-	x	x	x
Blast-Predator	10	x	x	x	x

Table 2. Experimental design to assess repeated exposures to BOP and stress on anxiety.

In the basic 4 x 4 balanced experimental design shown above rats in the control (sham) condition were exposed to the BOP device without experiencing the blast and will also be exposed to a sham predator environment without explicit exposure to the predators. Behavioral assessment on the EPM occurred on two occasions prior to the BOP, stress, or sham exposures and on two occasions 24 and 72 hours after the exposures occurred 24 hours after the last exposure to BOP/stress or sham condition(s).

The results of the experiment are provided below. Figure 2 depicts the difference scores in the respective conditions post exposure relative to the baseline performance. There were no significant statistical differences between the four treatment conditions. All of the groups demonstrated decreased activity on the first post-exposure day. It may be that greater statistical power to distinguish the conditions may have been reached with additional animals. In our experience however, treatment effects with group sizes of 10 are not likely to manifest with higher numbers of animals per group. Additionally, based upon our prior experience and power calculations, 10 animals per group are adequate to see treatment effects using this paradigm. I does not appear that BOP and stress exposures produced greater anxiety than the control groups. We speculate that the time soon after the blast and stress exposures represents a noisy environment where the ramifications of the exposures may not be manifest. In collaboration with our VA partners we have noted that indices of occur several months after exposure to blast overpressure. It may be that differences in these exposure conditions may not have been revealed until weeks after the experience if the underlying mechanisms take time. We plan to assess

the timeframe of assessment after blast and stress from hours to weeks and months to determine if the changes are a late manifestation of the exposures.

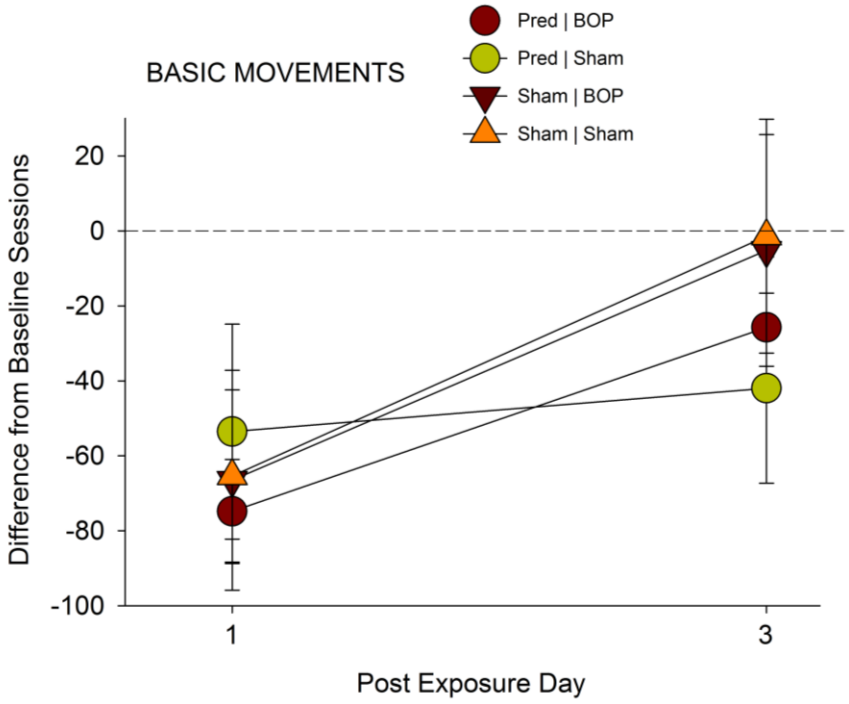

Figure 2. Effects of Repeated BOP and predator exposure on the Elevated Plus Maze performance of anxiety.

5. CONCLUSIONS:

- The results of this study did not demonstrate any differences on the acquisition of learning the spatial location or manifestation of anxiety for any of the experimental conditions leading to the conclusion that, at least within the parameters of the current study, the addition of a stressful experience to exposures to blast overpressure does not have an additive effect in impairing cognitive or emotive performance when the assessment occurs shortly after the exposures.

- The results of the biomarker studies will be reported in Dr. Genovese's report.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

None.

7. INVENTIONS, PATENTS AND LICENSES

None.

8. REPORTABLE OUTCOMES

None.

9. OTHER ACHIEVEMENTS

As part of collaboration we (Ahlers) published a paper examining the long-term effects of exposure to blast overpressure on the manifestation of anxiety behaviors several months after the blast exposure. The blast exposure parameters are similar to those employed in this study, however the studies are distinct in several ways. The primary emphasis of this effort is the near simultaneous exposure to blast and stress whereas the Elder et al. paper examined the effects of blast on anxiety behaviors 4 months or longer after the exposure to blast only (no stress exposures). The reference to the Elder paper is provided below.

10. REFERENCES

Elder, G.A., Dorr, N. P., De Gasperi, R., Gama Sosa, M. A., Shaughness, M. C., Maudlin-Jeronimo, E., Hall, A. A., McCarron, R. M., Ahlers, S. T. Blast Exposure Induces Post Traumatic Stress Disorder-Related Traits in a Rat Model of Mild Traumatic Brain Injury. *Journal of Neurotrauma*, 29:1-12, 2012

11. APPENDICES:

None.